REVIEW ARTICLE

Chagas' disease and Duffy antigen/receptor for chemokine (DARC): a mini-review

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Abstract: Duffy gene (*FY*) codifies the transmembrane glycoprotein Duffy (gp-Fy) of 35 to 43 kDa which is moderately immunogenic. This glycoprotein is polymorphic, and constitutes the antigens of the Duffy histo-blood system which were designated receptors for chemokines and denominated DARC (Duffy antigen/receptor for chemokine). This receptor has an important role in the regulation of chemokine levels in the circulation, as it binds and adsorbs them on the surface of red cells as a reservoir. It plays a "sink" role, which can contribute to homeostasis by removing inflammatory chemokines from circulation as well as maintaining them in plasmatic levels. Chronic Chagas' cardiopathy (CCC) is the most frequent form of the disease. It is an inflammatory disease, in which infiltrated inflammatory cells play an important role in the development and progress of the infection. High chemokine levels in the plasma have been associated with the disease severity in patients with heart failure. In this context, the profile of DARC expression could play an important function as a receptor for chemokines in Chagas' disease, in patients with CCC, as it can modulate damage from this inflammatory disease.

Key words: DARC antigen, Duffy blood-group system, Chagas' disease, Chagas' cardiomyopathy.

INTRODUCTION

The Duffy gene (*FY*) codifies the moderately immunogenic transmembrane glycoprotein Duffy (gp-Fy) of 35 to 43 kDa (1). This glycoprotein is polymorphic, and constitutes the antigens of the Duffy histo-blood system, which are codified by *FYA* and *FYB* alleles of the *FY* gene playing a co-dominant role. The *FYA* and *FYB* alleles differ due to substitution of the base G by another A in the 125 nucleotide, which develops a common polymorphism in the Caucasian population. This single nucleotide polymorphism (SNP) results in the substitution of amino acid glycine by asparagine in position 42 (2).

Although this polymorphism originates distinct glycoproteins called Fya and Fyb antigens, both have moderate immunogenicity. They can be identified with the use of anti-Fya and anti-Fyb anti-sera, allowing the characterization of four erythrocyte phenotypes: Fy(a+b–), Fy(a–b+), Fy(a+b+) and Fy(a–b–) (3, 4). Negative phenotype Duffy [Fy(a–b–)] is the result of a variant *FYB* allele *(FYB-33)*, which presents a single point mutation where there is a T to C substitution in nucleotide –33 (–33T>C), also known as the GATA-BOX, located in the promoter region of the *FY* gene (5, 6).

The differential distribution of DARC antigenic determinant between ethnic groups is a characteristic of this histo-blood system. As an example, *FYA* is prevalent in European, Chinese, Japanese, and Malaysian populations, but rarely in African population. And on the other hand, in Caucasians, *FYB* is widely found compared to Asian and African populations (7, 8). Homozygote

individuals for allele *FYB-33* (DARC negative) are common in African population, but rare in other ethnic groups (9). In Brazil, some studies on intra-ethnic genetic diversity in five geographic regions have shown some variations in the origin and ethnic composition of three subgroups (European, African, and mixed descendants) (10). Therefore, *FY* allele frequencies from distinct ethic groups are of great importance for phenotype and genetic composition distribution (11-15).

The Duffy antigen is expressed in erythrocytes, Purkinje cells of the cerebellum, and epithelial cells of the kidneys and lungs (1, 16-20). Erythrocytes with gp-Fy absence do not manifest alterations, therefore individuals with phenotype Fy (a–b–), who do not present antigen Duffy in their erythrocytes, are seemingly normal (21). The Duffy antigen was identified as a malaria parasite receptor, as individuals who do not express that protein in erythrocytes cannot be invaded by *Plasmodium knowlesi* or *Plasmodium vivax* (22, 23). Several studies in Brazil have demonstrated the association between the Duffy antigen and malaria by *P. vivax*; the first was by Colauto *et al.* (24). However, recent studies have verified infection by *P. vivax* in Fy (a–b–) individuals from Brazil and Eastern Africa (25-28). Genetic mechanisms for the Duffy-negative phenotype in erythrocytes have preserved its expression in the endothelium determining an important role in inflammation physiopathology (16, 19).

Antigens of the Duffy blood system were designated chemokine receptors and denominated Duffy antigen/receptor for chemokine (DARC), as a result of experiments performed in Duffy positive individuals, who absorbed interleukin-8 (CXCL8/IL-8) on the erythrocyte surface (1, 29-32).

Chemokines are small cytokines of 8 to 10 kDa that directly induce cell movement through the organism or induce specific functions in activated cells (33, 34). They are classified in subfamilies according to the number and location of the amino-terminal cysteine residues. Of these, two main subfamilies are highlighted: CC, in which the cysteine residues are adjacent, and CXC, in which these residues are separated by an amino acid (35, 36).

These chemokines can also be divided into inflammatory and homeostatic proteins, based on the conditions and production site (35-39).

Inflammatory (or induced) chemokines, such as CXCL8/IL-8, CCL5/RANTES, CCL11/eotaxin, CCL4/MIP-1 CCL2/MCP-1, and CXCL10/IP-10 are produced by several cells in response to inflammatory stimulus. They work in recruiting cells such as monocytes, granulocytes and T cells (effector) to inflammation sites. While homeostatic (or constitutive) chemokines are expressed constitutively, and they can be involved in both lymphoid cell organization and basal leucocyte traffic (36, 40).

IL-8 is involved in the monocyte and neutrophil recruitment and activation process for sites of acute inflammatory response. It presents certain longevity in these sites, being produced at the beginning of inflammatory response and is active for a long period of time; days and weeks. Several studies have been performed on IL-8 and cardiovascular diseases, some identified this chemokine in vascular injury sites, while others demonstrated that it plays a role in several phases of atherosclerosis, as either a marker or a potential therapeutic target (41). Experiments carried out by Kim *et al.* (42) have shown IL-8 as having considerable relevance in the pathogenesis of the hypertension. Simonini *et al.* (43) evaluated its participation in the angiogenic activity of the atherosclerosis. They concluded that IL-8 is an important angiogenesis mediator. Other authors have demonstrated that its neutralization significantly reduces the degree of necrosis in an animal model of myocardial ischemia-reperfusion injury (41, 44).

Unlike other chemokine receptors, DARC is a promiscuous receptor, because it interacts with both CC and CXC classes with high affinity, while most chemokine receptors link themselves to just one of the classes (31, 45-48). The Duffy antigen is a receptor for inflammatory chemokines. Experiments have demonstrated DARC has an affinity with the following 16 chemokines: CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL11, CCL2, CCL5, CCL7, CCL11, CCL13, CCL14, and CCL17 (49, 50). Immunohistochemical studies have demonstrated DARC expression on the surface of venule and small vein endothelial cells, considered important sites for recruiting leukocytes to inflammation areas, induced by chemokines and facilitating their movement through the endothelium of different tissues (16, 51, 52). Studies suggest DARC contribution in

chemokine transcytosis from the intravascular to the extravascular space, favoring the migration of leukocytes involved in inflammation (50, 53). DARC plays an important role in the regulation of circulation chemokine levels, as it binds to and adsorbs them on the surface of red cells, as a chemokine reservoir performing a "sink" role (29, 50). Therefore, DARC can collaborate with homeostasis in the removal of inflammatory chemokines in the circulation, thus avoiding the loss of these inflammatory mediators for organs and distant tissues, as well as maintaining them in plasma levels, due to subsequent liberation from the erythrocyte surface (50, 54). However, the theory that DARC plays a "sink" function has been questioned. Some authors have demonstrated that there is no chemokine intracellular variation associated to DARC (55). Thus, further investigations are needed to better understand this role of DARC.

DARC is involved in several "classic" chemokine receptor diseases such as giant cell arteritis, renal diseases and transplantations, as well as during acute transplant rejection (56, 57-60). Susceptibility to asthma was correlated with the absence of DARC expression in red cells from certain Afro-descendant populations (61). A recent study showed that the absence of DARC in erythrocytes resulted in a 40% increase in the risk of acquiring HIV; however, these individuals presented a short-term progression of the disease (55, 62). Epidemiological data suggest that DARC absence in the erythrocyte has contributed to an increase in prostate cancer incidence and mortality (46, 63). DARC expression in cancerous lung cells may be associated with decreased tumor vascularization and a reduction in metastatic potential, as well as a decrease breast cancer cell growth, due to sequestration of angiogenic chemokines and inhibition of tumor vascularization (64, 65).

DARC AND CHAGAS' DISEASE

Chagas' disease was described by the great scientist Carlos Chagas in 1909. It is a parasitic disease that occurs particularly in America. Its etiological agent is the flagellated protozoan *Trypanosoma cruzi* belonging to the phylum Protozoa, order Kinetoplastida, family Trypanosomatidae and the gender *Trypanosoma* (66, 67). The main transmission form to the human host is by insects of the order Hemiptera, family Reduviidae and genera *Triatoma, Panstrongylus* and *Rhodnius* (68, 69). Other transmission mechanisms such as blood transfusions and congenital transmission have also been reported in mainly urban areas and non-endemic countries (70). Nearly 15 to 16 million people are infected by *T. cruzi* in Latin America (71).

Inflammatory cytokines play a central role in infection by *T. cruzi*. The acute phase of Chagas' disease is characterized by an exacerbated production of inflammatory cytokines, including IL-12, TNF-α, and IFN-γ, and chemokines such as CCL2, CCL3, CCL4, CCL5, and CXCL10 (72, 73). CCL5 and CCL2, which bind to the Duffy antigen, have been singled out from these inflammatory mediators.

After the acute phase, most individuals are asymptomatic as they present the indeterminate form of Chagas' disease, while between 30 to 40% of parasitized individuals can develop heart, digestive, or mixed problems. The most frequent form, inflammatory myocardiopathy also known as chronic chagasic cardiopathy (CCC) accounts for 20 to 30% of individuals (74).

CCC is an inflammatory disease, characterized by the presence of diffuse myocarditis with a remodeling process of intense myocardial fibrosis, hypertrophy, and lesion of cardiac muscular fibers (75, 76). Clinical data suggest that the inflammatory infiltrate plays an important role in the development and progression of Chagas' disease, as mononuclear infiltrate is associated with greater cardiomyocyte destruction and local fibrosis in CCC (76, 77). Development of this myocardiopathy involves three possible pathogenic mechanisms: cardiac dysautonomia, microcirculation changes, and tissue damage resulting from the inflammatory and immune responses (73).

The chronic phase of the disease also presents high inflammatory cytokine production, possibly due to longer exposure to the parasite. An increase in plasmatic TNF-α and IFN–γ levels occurs; this is also observed in patients with the indeterminate form of the disease. Individuals with CCC present high TNF-α and CCL2 levels in the circulation in relation to those with the indeterminate form (73). It is important to point out the inflammatory chemokines whose receptor is DARC; these include CXCL8/IL-8, CCL5/RANTES, CCL11/eotaxin, and CCL2/MCP-1.

High chemokine concentrations in plasma have been associated with disease severity in patients with CCC, for example high levels of CCL2 and TNF-α, the latter directly correlating with the degree of heart failure in these patients (78). Experiments carried out by Cunha-Neto *et al.* (79) suggest that IFN-γ and CCL2 are related to gene expression of the cardiomyocytes involved in the pathological hypertrophy process. Moreover, the intensity of acute and chronic myocarditis in mice (C3H/He) infected with Colombian strain was directly associated with CCL2 concentration in the heart (80).

High expression of CCR5 (receptor for CCL3, CCL4, and CCL5) was detected in leukocyte patients with chagasic cardiomyopathy. Polymorphism in the promoter region of the *CCR5* gene (*CCR5* 59029 A→G), associated with lower CCR5 expression in leukocytes was more frequent in asymptomatic patients than those with chagasic cardiomyopathy (78, 81). Others studies have shown that mice lacking the CCR5 receptor present a significant reduction of cardiac inflammatory infiltrate, suggesting the importance of this receptor in lymphocyte migration and control of local parasite replication (82). It is important to emphasize that DARC presents a significant homology with receptor CCR5, and it is also a receptor for chemokine CCL5 (55).

Studies carried out by Damås *et al.* (83) have demonstrated the presence of CCL2 and CXCL8 in cardiomyocytes. This suggests the important role these cells play in the inflammatory process, either by chemokine production, or by expressing their receptor. High chemokine expression and corresponding receptors in the myocardium and circulating leukocytes suggests a relevant function for these mediators in various forms of myocardial failure (84). Experiments carried out with patients who presented with CCC revealed high chemokine expression in heart tissue. Other data suggest that local production of these inflammatory mediators can perform a highly important function in the heart damage observed in CCC (85).

CONCLUDING REMARKS

Although investigated in several studies, the actual role of chemokines in myocardium disease is not completely explained. In this context, the profile of DARC expression plays an important role as a chemokine receptor in Chagas' disease patients with CCC. It can modulate damage by this inflammatory disease. However, many aspects of Duffy antigen biology should be considered when determining their effective functions, because despite all the knowledge on the relationship between DARC structure/function and tissue location, its effective role still remains uncertain in normal and damaged physiology.

CONFLICTS OF INTEREST
There are no conflicts of interest.

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